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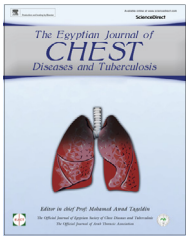

ORIGINAL ARTICLE

Assessment of eotaxin 1 in exhaled breath condensate of chronic obstructive pulmonary disease patients

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KEYWORDS

COPD;
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Abstract The aim of this work was to assess eotaxin 1 level in exhaled breath condensate of exacerbated and stable COPD patients in relation to normal subjects.

Subjects and methods: There were 16 COPD patients (during infective exacerbation), 16 stable COPD patients and 20 healthy volunteers as controls matched with them in age, sex and smoking history. EBC was collected and concentration of eotaxin 1 was measured by using Human Eotaxin 1 ELISA Kits.

Results: The mean eotaxin 1 concentration in exhaled breath condensate of studied groups was 962.5 ± 150 pg/ml in the exacerbated COPD group, 427.8 ± 186.6 pg/ml in the stable group and 89.2 ± 47.5 pg/ml in the control group. Age, smoking, FVC, FEV1, and FEV1/FVC, showed no significant correlation with eotaxin 1 levels among all the studied groups.

Conclusion: Eotaxin 1 levels in exhaled breath condensate of COPD patients during infective exacerbation was significantly higher than in stable COPD patients and both groups showed significant higher levels than the control group.

The prognosis for chronic obstructive pulmonary disease (COPD) is adversely affected by acute exacerbation, which increases mortality and morbidity and causes an irreversible decline of pulmonary functions [1]. It has been suggested that patients with more frequent exacerbations have an increase in airway inflammation and a higher baseline sputum interleukin (IL)-6 and IL 8 levels, and these cytokines may predict the frequency of future exacerbations [2]. It has also been reported that not only neutrophils but also eosinophils and lymphocytes may participate in the increased airway inflammation during acute exacerbation [3]. However, the details of the mechanism of increase in airway inflammation during exacerbation remain obscure.

Abbreviations: COPD, chronic obstructive pulmonary disease; EBC, exhaled breath condensate; IL, interleukin; Th, T-helper cells; FVC, forced vital capacity; FEV1, forced expiratory volume in first second; CCR3, c chemokine receptor 3

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It has been suggested that, among various causes of acute exacerbation, respiratory tract infection due to virus or bacteria may account for as much as, or even more than 80% of COPD exacerbations [4]. Infection of airway epithelial cells induces various cytokines and chemokines, such as eotaxin 1 which is a potent and selective chemoattractant for human eosinophils .The human eotaxin receptor, CCR3 is expressed on eosinophils, basophils and Th2 cells [5].

Eotaxin, in association with Th1-derived cytokine IL2 and Th2 – derived cytokine IL4 is an important T lymphocyte activator, stimulating the directional migration, adhesion, accumulation, and recruitment of T lymphocytes in parallel with the accumulation of eosinophils and basophils during the process of certain types of inflammation [6].

Exhaled breath condensate (EBC) is a promising source of biomarkers of lung disease. It is important to note that EBC is not a biomarker, but rather a matrix in which biomarkers may be identified, in that way equivalent to blood, sweat, tears, urine and saliva. EBC may be thought of either as a body fluid or as a condensate of exhaled gas [7].

Eotaxin 1 was assessed by many studies in patients with bronchial asthma. It was measured in serum and EBC of stable and unstable asthmatics and correlated with the level of control of asthma and monitoring of inflammatory process [8,9].

Many studies have investigated eotaxin 1 in serum and sputum of COPD patients, however, up to our knowledge no studies have investigated eotaxin 1 in exhaled breath condensate of COPD patients.

So, the aim of this work was to assess eotaxin 1 level in exhaled breath condensate of exacerbated and stable COPD patients in comparison to normal subjects.

Subjects and methods

This study was conducted in the chest department and outpatient clinic of the Ain Shams University hospital in the period between February and April 2013. Fifty-two individuals were included in the study; they were 32 COPD patients (diagnosed according to GOLD 2011 guidelines) and 20 controls. The candidates were classified into: 16 COPD patients (during infective exacerbation), 16 stable COPD patients and 20 healthy volunteers as controls matched with them in age, sex and smoking history.

Exclusion criteria

The following patients were excluded from the study:

- Patients with history of bronchial asthma.
- Patients with history of intake of inhaled or oral steroids.
- Patients with heart failure.
- Patients with pulmonary thromboembolism.

All candidates were subjected to:

- Full history taking.
- Thorough clinical examination.
- Chest X-ray.
- Routine laboratory investigations.
- Pulmonary spirometry to measure: FEV1, FVC and FEV1/FVC ratio. It was done using a Flowmate spirometer.
- EBC was collected by using a commercially available condenser (EcoScreen; Jaeger, Würzburg, Germany) according to the current ATS/ERS guidelines [10]. Patients were instructed to breathe tidally for 10 min with nose clip. The respiratory rate ranged from 15 to 20 breaths/min. Patients were asked to swallow their saliva periodically and to temporarily discontinue collection if they need to cough. At the end of collection 1.5–3.5 ml aliquots of condensate were transferred to Eppendorf tubes and immediately frozen. Samples were stored at −20 °C [11]. Concentration of eotaxin 1 was measured by using Human Eotaxin 1 ELISA Kits.

Statistical analysis

Continuous variables are expressed as mean and standard deviation. Categorical variables are expressed as frequencies and percents. A significance level of $P < 0.05$ was used in all tests. All statistical procedures were carried out using SPSS version 15 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The current study was conducted on 52 male subjects, they were divided into: 16 exacerbated COPD patients, 16 stable COPD patients and 20 healthy volunteers as the control group. Their mean age was (59.5 ± 11.6, 56.9 ± 12.0 and 53.2 ± 5.2) years, respectively, with no statistical significant difference.

Among the exacerbated group 14 patients out of 16 (87.5%) were current smokers and two patients (12.5%) were ex-smoker with a mean smoking index 36.8 ± 16.8 pack year. While in the stable group 10 patients out of 16 (62.5%) were smokers, 5 (31.3%) patients were ex-smokers and one patient out of 16 (6.3%) was a non-smoker with a mean smoking index 42.4 ± 17.6 pack-year. In the control group there were 18 out of 20 (90%) current smokers, one ex-smoker (5%) and one non-smoker (5%). There was no statistical significant difference between the studied groups as regard smoking habit.

The spirometry showed no statistical significant difference between the exacerbated and stable COPD group as the mean FVC was 64.5% ± 21.8 and 64.2% ± 20.7 of the predicted in exacerbated and stable COPD patients, respectively. The mean FEV1 was 48.0% ± 19.8 and 47.6% ± 20.9 in exacerbated and stable groups, respectively. Also the mean FEV1/FVC ratio was 58.2% ± 10.6 and 58% ± 9.9 in exacerbated and stable groups, respectively.

As regard eotaxin 1 concentration in exhaled breath condensate of studied groups it was 962.5 ± 150 pg/ml in the exacerbated COPD group, 427.8 ± 186.6 pg/ml in the stable group and 89.2 ± 47.5 pg/ml in the control group.

Table 1 shows no significant difference between exacerbated and stable COPD cases as regard age, smoking habits, and spirometry. However, a highly significant difference between exacerbated and stable COPD cases was present as regard eotaxin 1 level with higher level among the exacerbated group.

Table 2 shows no significant difference between exacerbated and controls as regard age and smoking habits. However, a highly significant difference between exacerbated

Table 1 Comparison between exacerbated and stable as regard personal and medical data.

		Group				t/x^2	P	Sig
		Exacerbated		Stable				
		Mean	±SD	Mean	±SD			
Age years		59.5	11.6	56.9	12.0	.614	.544*	NS
Smoking	Smoker	14	87.5%	10	62.5%	2.800	.220**	NS
	Ex smoker	2	12.5%	5	31.3%			
	Non smoker	0	0%	1	6.3%			
Smoking index pack/yr		32.8	15.6	42.4	17.6	–1.410	.173*	NS
FVC		64.5	21.8	64.2	20.7	.039	.969*	NS
FEV1		48.0	19.8	47.6	20.9	.058	.954*	NS
FEV1FVC		58.2	10.6	58.0	9.9	.058	.954*	NS
Eotaxin 1 conc.		962.5	150.0	427.8	186.6	8.934	.0001*	HS

* Student t test.
** Chi square test.

Table 2 Comparison between exacerbated and control as regard personal and medical data.

		Group				t/x^2	P	Sig
		Exacerbated COPD		Control				
		Mean	±SD	Mean	±SD			
Age years		59.5	11.6	53.20	5.217	2.011	.058*	NS
Smoking	Smoker	14	87.5%	18	90.0%	1.425	.768**	NS
	Ex smoker	2	12.5%	1	5.0%			
	Non smoker	0	0%	1	5.0%			
Eotaxin 1 conc.		962.5	150.0	89.2	47.5	24.6	.0001*	HS

* Student t test.
** Chi square test.

Table 3 Comparison between stable and control as regard personal and medical data.

		Group				t/x^2	P	Sig
		Stable COPD		Control				
		Mean	±SD	Mean	±SD			
Age years		56.9	12.0	53.2	5.2	1.162	.259	NS
Smoking	Smoker	10	62.5%	18	90.0%	4.56	.069	NS
	Ex smoker	5	31.3%	1	5.0%			
	Non smoker	1	6.3%	1	5.0%			
Eotaxin 1 conc.		427.8	186.6	89.2	47.5	7.83	.0001	HS

and controls was present as regard eotaxin 1 level with higher level among the exacerbated group.

Table 3 shows no statistical significant difference between stable and controls as regard age and smoking habits. However, a highly significant difference between stable and controls was present as regard eotaxin 1 level with higher level among the stable group. That is to say there was significant difference in the level of eotaxin 1 concentration in EBC of studied groups showing the highest level in the exacerbated group and lowest in control group (Fig. 1).

Age, smoking, FVC, FEV1, and FEV1/FVC, showed no significant correlation with eotaxin 1 levels among all the studied groups.

Discussion

To our knowledge, this is the first time eotaxin 1 level was assessed in exhaled breath condensate of exacerbated and stable COPD patients.

Many reports have studied eotaxin 1 level in serum, sputum and exhaled breath condensate of stable and unstable asthmatic patients. Other reports studied eotaxin 1 levels in serum and sputum of COPD patients, but not in exhaled breath condensate. So we faced lack of data regarding eotaxin 1 in exhaled breath condensate of COPD patients to compare our results with these data.

In the current study we aimed to assess eotaxin 1 level in exhaled breath condensate of COPD patients during infective exacerbation, and stable COPD patients in comparison to the control group.

Our study documented that eotaxin 1 level in exhaled breath condensate of COPD patients during exacerbation was significantly higher than that of the control group. This coincides with Fujimoto et al. [12] who investigated many inflammatory cells such as lymphocytes, neutrophils, and eosinophils and mediators such as interleukin-8 and eotaxin in sputum of COPD patients during acute exacerbation and found a significant difference in these cells and mediators

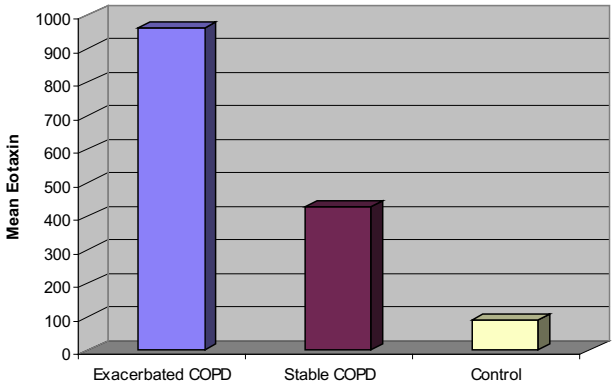

Figure 1 A significant difference in the level of eotaxin 1 concentration in EBC of studied groups showing the highest level in the exacerbated group and lowest in control group.

including eotaxin between COPD patients and the control group.

In the current study the level of eotaxin 1 in EBC of stable COPD was significantly higher than that of the control group, however it is still lower than the level in the exacerbated group.

This agree with Rozyk et al. [13] who studied the serum eotaxin and serum eosinophil cationic protein in asthmatic and COPD patients and found that serum eotaxin is significantly higher in COPD patients than in asthmatic patients or the control group.

This also coincides to some extent with Fujimoto et al. [12] who investigated eotaxin level (among other mediators and inflammatory cells) in sputum of stable and acutely exacerbated COPD patients, and stated that the eotaxin level in sputum from COPD patients in a stable phase was significantly higher than that from the age-matched control group. On the other hand, they disagree with our results in that they found no significant difference between exacerbated and stable COPD patients as regard eotaxin level in sputum.

In another study to investigate whether eosinophils and T lymphocytes may be indicators of disease stability in COPD patients, the researchers studied 45 COPD patients with moderately severe airway obstruction and 11 controls. Lung lavage and plasma samples were examined for eotaxin and other cytokines. The team found that lung lavage eotaxin 1 levels were significantly higher in patients with rapidly progressing COPD than in stable COPD patients and controls. They also found that, in contrast, patients with stable COPD had significantly lower plasma levels of eotaxin 1 than both rapid decliners and controls, who had the highest levels [14].

Thus, all these results including ours, suggest that eotaxin may participate in eosinophilic inflammation as eosinophil chemoattractants in COPD patients during exacerbation.

In the current study there was no statistical significant difference between the studied groups as regard the smoking habit. This coincides with another study [15] which was done to compare the eosinophil and neutrophil chemotactic activity contained in EBC from healthy subjects and patients with COPD. They stated that smoking did not influence eosinophil chemotactic activity in healthy subjects or patients with COPD.

On the other hand, Zhu et al. [16] who studied the gene expression of interleukin-4, interleukin-5 eosinophil chemoattractant in exacerbation of bronchitis, found that expression of eotaxin did not show any significant change in smokers with chronic bronchitis than in healthy non smokers. Whereas Fujimoto et al. [12] found that eotaxin in the supernatant of sputum from patients with both stable and unstable COPD groups in a stable phase was significantly higher than those in healthy non smokers.

However, in our study there were only two non smokers, one of them in the stable COPD group and the other was in the control group and all other candidates were smokers or ex-smokers to keep matching between studied groups as regard smoking habit. Thus it is difficult for us to compare between smokers and non smokers.

In the current study FVC, FEV1 and FEV1/FVC, showed no significant correlation with eotaxin 1 levels among all the studied groups. These results agree with Fujimoto et al. [12] who found no significant difference between stable COPD, unstable COPD and non smokers groups as regard pulmonary functions in correlation with eotaxin levels.

However, these results disagree with another study [13] that investigated eotaxin in serum of patients with asthma or chronic obstructive pulmonary disease and reported that serum eotaxin levels were inversely related to the lung function (FEV1) in patients with asthma or COPD.

In conclusion, we found significantly higher eotaxin 1 levels in exhaled breath condensate of exacerbated and stable COPD patients in comparison with the control group with much higher levels in the exacerbated group. These results support the reports suggested that not only neutrophils but also eosinophils and lymphocytes may participate in the increased airway inflammation during acute exacerbation. Thus, the low molecular weight compounds that have been developed and can block the eotaxin receptors CCR3 and prevent stimulation by eotaxin, may provide the potential for oral drugs that can prevent eosinophil recruitment into the lung and the associated damage and dysfunction. These drugs may provide a promising way to control exacerbations and/or decrease their frequencies.

Conflict of interest

No conflict of interest.

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